THE CAUSATION OF MATURATION IN THE EGGS OF LIMPETS BY CHEMICAL MEANS.[1]

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In his experiments on the maturation of the eggs of the starfish (*Asterias forbesii*)[2] Dr. Loeb found that the eggs when removed from the ovaries of the animal are in most cases immature, but that if they come in contact with sea water, during the breeding season they begin to maturate. The immature state is characterized by a large, plainly-visible nucleus, which, during maturation, becomes invisible. This process of maturation is completed in from one to two hours after the eggs are removed from the ovaries and placed in sea water. Only when the process of maturation is completed is it possible to fertilize the eggs with sperm.

Experimentation on the maturation of these eggs showed that the chemical conditions necessary to cause or accelerate the maturation processes are, that there must be present in the sea water two substances, free oxygen and hydroxyl ions of a certain concentration. Dr. Loeb found further that if, upon becoming mature the eggs were not caused to develop by the addition of sperm, then they died very rapidly. The change in the appearance of the egg after death is very marked; the living egg having a light yellow color, after death it becomes black; and where in the living egg the protoplasm is homogeneous and somewhat transparent, in the dead egg it becomes granular and opaque. Thus it was found that in a culture that had been standing for twenty-four hours, all the eggs that had remained immature were alive, and all those that had matured were dead. This shows that the mature eggs of the starfish die in the course of a few hours, while under exactly the same conditions the immature eggs remain alive.

When the maturation is prevented artificially through lack of

[1] From the Herzstein Research Laboratory of the University of California.

[2] BIOLOGICAL BULLETIN, Vol. 3, No. 6, Nov., 1902.

oxygen or by the addition of either acid or potassium cyanide to the sea water the eggs remain alive a considerable period of time. The eggs in which maturation has already begun, or has just been completed, are also saved from rapid death by these means.[1]

Later, he found that the eggs of a mollusc (*Lottia gigantea*) when removed from the ovary were always immature and would remain so even if they were kept in water for two days. Such eggs could not be fertilized by sperm. But if they were first treated with a mixture of 50 c.c. sea water and 1 c.c. 1/10 *n* NaHO for from four to five hours maturation took place with the result that over 75 per cent. of the eggs would develop into larvæ in normal sea water after the addition of sperm. He found also that treatment with sea water containing benzol would cause the eggs to become mature very rapiply.[2]

As in the case of the starfish egg the maturation processes in the eggs of *Lottia*, induced by alkali or benzol can be prevented by lack of oxygen, or by either the addition of acid or potassium cyanide to the sea water.

The fact that the immature eggs of the starfish and especially those of *Lottia* could be caused to become mature in this way, suggested experimentation upon the other limpets found at Pacific Grove. Four varieties of *Acmæa* were used by the writer. *Acmæa patina*, *pelta*, *persona* and *scabra*. The eggs of the *Acmæa* studied are all immature when removed from the ovaries. They look very much like those of *Lottia* (Fig. 1) and are characterized by a greenish color, a very irregular outline, and a transparent membrane — a chorion which conforms to the shape of the egg. In maturing the chorion disappears and the egg becomes perfectly spherical in shape and rather more opaque, Fig. 2. Only when this maturation process is completed is it possible to fertilize the egg by the addition of sperm. The eggs of *Acmæa* remain immature in sea water for two or three days and only occasionally does one find any mature eggs in the culture that has stood for any length of time.

To determine, whether by increasing the alkalinity of the sea

[1] These experiments have recently been repeated and confirmed by A. P. Matthews, *Am. Journal of Physiology*, 1907.

[2] *Univ. of Cal. Publications*, Nov. 17, 1905, Vol. 3, No. 1, pp. 1–8.

water the eggs would become mature, I subjected the eggs to the following solutions: 50 c.c. sea water, plus 0.4 c.c.; 0.7 c.c.; 1.0 c.c.; 1.5 c.c. 1/10 *n* NaHO for 1, 1.5, 2 and 3 hours. After each period the eggs were transferred to normal sea water

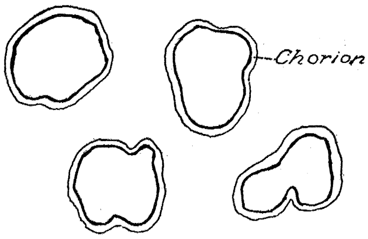

FIG. 1.—Immature eggs.

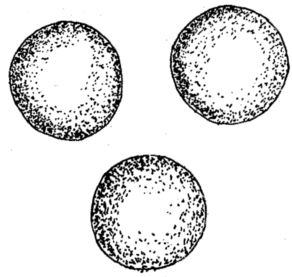

FIG. 2.—Mature eggs.

and sperm added about fifteen minutes later. At the end of two hours, maturation in most of the cases, was completed. Eighteen hours later the following results were obtained:

TABLE I.

Solution.	Length of Treatment with Alkaline Sea Water.			
	1 Hour.	1.5 Hours.	2 Hours.	3 Hours.
50 c.c. sea water + .4 c.c. *n*/10 NaHO.	10 % mature. 1 swimming larva. Some disintegrated eggs.	15 % mature. All swimming.	40 % mature. 10 % swimming. Rest disintegrating.	40 % mature. 5 % swimming. Rest disintegrating.
50 c.c. sea water + .7 c.c. *n*/10 NaHO.	30 % mature. 15 % larvæ. Few disintegrating.	50 % mature. 40 % swimming.	70 % mature. and swimming. 40 % irregular.	90 % mature and swimming. 10 % disintegrating.
50 c.c. sea water + 1 c.c. *n*/10 NaHO.	40 % mature. All swimming.	90 % mature and swimming. Few disintegrating.	90 % swimming. 40 % irregular.	98 % mature and swimming. 10 % disintegrating.
50 c.c. sea water + 1.5 *n*/10 NaHO.	All mature and swimming. Fine larvæ.	98 % mature and swimming. Few disintegrating.	95 % mature and swimming. 20 % irregular. Disintegration.	95 % swimming. 5 % disintegrating.

From the table we see that the greatest number of the eggs become mature and form larvæ when 1.5 c.c. *n*/10 NaHO are added to 50 c.c. sea water, and the eggs subjected to this mix-

ture for one hour. Practically all become mature and the larvæ resulting are excellent.

If, however, the eggs are under-exposed or over-exposed to the solutions they either break up or segment irregularly and it is not very long before these latter eggs too, break up, and disintegrate. The number of disintegrating eggs is smaller when the eggs are treated for one hour with the mixture of 50 c.c. sea water and 1.5 c.c. *n*/10 NaHO than with any of the other solutions used. When sperm was added to the eggs which had not been treated with alkaline sea water, not a single egg became mature or developed into a swimming larva.

Next I tried the effects of lack of oxygen on maturation. The alkaline sea water (50 c.c. sea water + 1.5 c.c. *n*/10 NaHO) was placed in a bottle and connected with the hydrogen generator, and a stream of hydrogen was passed through the solution for two hours. The eggs were then quickly introduced and left for one hour in the solution, with a good stream of hydrogen still passing through. The eggs were then transferred to normal sea water and sperm added. The following table shows the result :

TABLE II.

Solution.	Treatment for 1 hr.
50 c.c. sea water + 1.5 c.c. *n*/10 NaHO with oxygen.	All mature and swimming. Few eggs disintegrating.
50 c.c. sea water + 1.5 cc. *n*/10 NaHO without oxygen.	About half a dozen mature eggs. Two larvæ. No disintegration.

This shows that, as in the case with *Lottia*, the alkali can only cause the maturation of the eggs of *Acmæa* if oxygen be present. If the eggs that had been kept from becoming mature by lack of oxygen were subsequently treated with akaline sea water in the presence of oxygen, practically all became mature, and would, upon the addition of sperm, develop into larvæ.

By stopping the oxidative processes in the eggs through the presence of potassium cyanide, maturation can also be inhibited. Thus, if to alkaline sea water a little potassium cyanide be added no maturation occurs.

TABLE III.

Solution.	Treatment for 1 hr.
50 c.c. sea water + 1.5 c.c. *n*/10 NaHO.	All mature and swimming. Little disintegration.
50 c.c. sea water + 1.5 c.c. *n*/10 NaHO + 1.2 c.c. 1/10% KCN.	Not one mature egg, in whole culture. No eggs disintegrated.

The processes which were accelerated by the presence of the alkali were completely inhibited by the potassium cyanide and it is especially to be noted that while disintegrated eggs were found in every case in the absence of potassium cyanide, no disintegrated eggs were found when potassium cyanide was present.

If the eggs that had been prevented from becoming mature through the presence of the potassium cyanide, were afterwards treated with alkaline sea water, containing no potassium cyanide, practically all became mature and formed larvæ when sperm was added. This shows further that no permanent injury is done to the eggs by treating them with KCN or by depriving them of oxygen for so short a period of time.

I next tried the effect of fat solvents, such as benzol, chloroform, ether and ethyl acetate, upon the immature eggs to see if by treatment with these substances maturation could be produced, and I found that in every case I got positive results. The method of procedure in general was as follows: 30 c.c. sea water were heated to 45° C. and a known quantity of the solvent was added and the mixture vigorously shaken to ensure complete solution. If the solution is not complete the eggs that come in contact with the droplets of the solute are immediately killed. The solution was then cooled down to 26° C., and the eggs introduced. At definite periods the eggs were removed to normal sea water and sperm added. Eighteen hours after the results obtained in Table IV were noted.

The best results were obtained when the eggs were exposed to the mixture for one minute; 85 per cent. of the eggs developing into larvæ upon the addition of sperm. If the eggs are exposed longer to the solution they become mature, but a large number segment irregularly. These irregularly segmenting eggs finally disintegrate before they reach the larval stage, or in the early larval stage. By this method of treatment the eggs become

mature much more rapidly than by the treatment with alkali. Usually from three fourths to one hour after treatment the eggs will be mature, whereas when the eggs are simply treated with the alkaline sea water maturation rarely takes place in less than two hours.

TABLE IV.

Solution.	Treatment for				
	½ Minute.	1 Minute.	1.5 Minutes.	2 Minutes.	2.5 Minutes.
30 c.c. sea water + 2 drops benzol.	25 % mature. 20 % swimming. Good larvae.	90 % mature. 85 % swimming. Good larvae. Little disintegration.	95 % mature. 50 % swimming. More disintegration.	90 % mature. 60 % swimming. Great many disintegrated eggs.	90 % mature. 60% swimming. 30 % disintegrated.

The maturation induced by the treatment with benzol is also an oxidative process, since it does not occur when the oxidations are prevented in the egg. The addition of 1 c.c. 1/10 per cent potassium cyanide to the sea water inhibits the maturation of the eggs treated with benzol, and it is especially noteworthy that not a trace of disintegration is present in the eggs that were overexposed to the solution in the presence of potassium cyanide. With the other fat solvents I have not as yet been able to get such a large percentage to develop as the table below will show.

TABLE V.

Solution.	Exposure.		
	¾ Minute.	1.5 Minutes.	2.5 Minutes.
30 c.c. sea water + 2 drops chloroform.	10 % swimming.	20 % swimming.	25 % swimming. Many eggs killed.
30 cc. sea water + 4 drops *n*/10 ether.	No larvæ.	5 % swimming.	10 % swimming
30 c.c. sea water + 6 c.c. *n*/2 ethyl acetate.	20 % larvæ.	40 % larvæ.	50 % larvæ.

These last experiments were not worked out as thoroughly as those with benzol on account of the lack of material, but we can readily see that all the solutions tried give positive results.

Conclusions.

1. As in the case of *Lottia* so also in that of *Acmæa patina*, *pelta*, *persona* and *scabra*, the addition of a small quantity of alkali to the sea water will cause the eggs to become mature.

2. The presence of oxygen hastens the destruction of the mature unfertilized eggs of *Acmæa*.

3. Lack of oxygen or the inhibition of the oxidative processes in the egg by the addition of a little potassium cyanide to the sea water not only prevents maturation but prevents also the disintegration of the mature unfertilized egg.

4. The treatment of the immature eggs of *Acmæa* with fat solvents, such as benzol, chloroform, ether, and ethyl acetate will also cause them to become mature.